Introduction: It has been reported that the primary infection rate in total hip arthroplasty is approximately 0.5–2.3% and the re-infection rate after revision of infected hip artificial joint is 21% [1, 2]. In particular, the infections caused by methicillin-resistant Staphylococcus aureus (MRSA) associated with the orthopaedic devices are a significant clinical problem [3]. Moreover, in the case of biofilm forming bacteria, the treatment is more difficult. The bacteria in biofilm are often less responsive to antibiotic treatment and host defense mechanisms [4].

We have developed the novel thermally-sprayed silver (Ag) containing hydroxyapatite (HA) coating onto the surface of implants in order to prevent adhesion of bacteria before forming their biofilm. The purpose of this study is to evaluate the antibacterial properties of Ag-HA coating in terms of inhibiting growth and bacterial adherence of MRSA.

Materials and Methods: HA powder containing 3wt % of silver oxide was sprayed on surface of titanium disks by the flame spraying, which is a kind of thermal spraying method using acetylene torch. HA powder without silver oxide was also flame sprayed to another disks for the control in this study.

(Antibacterial activity test) The strain of bacteria used in this study was biofilm forming MRSA (BF-MRSA) (UOEH6 strain from University of Occupational and Environmental Health). The 1 ml of this cell suspension (TSB with 1.0% glucose (Antibacterial activity test) The strain of bacteria used in this study was biofilm forming MRSA (BF-MRSA) (UOEH6 strain from University of Occupational and Environmental Health). The 1 ml of this cell suspension (TSB with 1.0% glucose) was used to regulate the viable bacterial counts to 10^5 colony forming units (CFU) before forming their biofilm. The purpose of this study is to evaluate the antibacterial properties of Ag-HA coating in terms of inhibiting growth and bacterial adherence of MRSA.

The antibacterial activity test was performed by the plate counting method using agar plate method. After the incubation, the number of viable bacteria was counted by agar plate method. The antibacterial activity of Ag-HA coating was compared with that of HA coating.

(Bacterial adhesion property) The disks rinsed with PBS as mentioned above were observed by light microscopy. The surfaces of the disks were subjected to SEM (scanning electron microscopy) observation.

Results: The titanium disks were able to be coated uniformly not only with HA but also with Ag-HA by the flame spraying method. Neither void nor crack was observed on the surfaces of the obtained coatings.

(Antibacterial activity) Fig. 1 shows the number of adhesive viable bacteria on the surfaces of Ag-HA and HA coatings. The surface bacterial adhesion was drastically decreased on the Ag-HA coating compared with the HA one; the number of adhesive viable bacteria in the Ag-HA was less than 10% of that in the HA.

(Bacterial adhesion property) Fig. 2 shows the light microscopic images of the Ag-HA and HA coatings after bacteria cultivation. The white-colored areas on the disks correspond to the biofilm by BP-MRSA. The coverage of the biofilm on the Ag-HA coating obtained by image analysis was about 10% of that on the HA coating. This result was approximately consistent with that of antibacterial activity test. The SEM images of the disks are shown in Fig. 3. Though the surface of the HA coating was fully covered by thick bacteria layer, only discrete bacteria were observed on the Ag-HA coating. This observation corresponded the results shown in Figures 1 and 2.

It was confirmed from both antibacterial activity and bacterial adhesion tests that the newly developed Ag-HA coating has an antibacterial activity for BF-MRSA.

Discussion: In recent years there have been several advances in antibacterial technology. So far, some antibacterial surfaces with silver-containing hydroxyapatite coating have been made by sputtering, sol-gel coating and other methods [5, 6]. But for last 20 years, however, HA coating of orthopaedic and dental implant surface was made by thermal spraying technology. It's widely used in clinical location.

In this study, we examined the antibacterial activity of the newly developed coating by thermal spraying technology in vitro. The Ag-HA coating showed 10 times higher antibacterial activity to BF-MRSA than HA as a control. The antibacterial activity is arisen from released Ag ion from the coating. The antibacterial properties of the Ag have been exploited for a long time in the medical field. The significant features of Ag are its wide spectrum of antibacterial susceptibility, low incidence of resistance, and the ability to inhibit bacterial colonization [7].

The number of BF-MRSA adhered to the material surface was much smaller in the Ag-HA than in the HA. It is expected that the Ag-HA coating utilizing thermal spraying technique could be useful for artificial joints with not only antibacterial activity but also osteoconductivity.